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论著

shRNA 介导GSTP1 基因沉默对激素非依赖性前列腺癌细胞株DU145 的影响

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摘要： 目的:探讨谷胱甘肽巯基转移酶P1 (glutathione S-transferase P1,GSTP1) 基因沉默对雄激素非依赖性前列腺癌细胞株DU145 增殖活性和对化学治疗药物敏感性的影响。方法:根据选取靶序列形成短发卡状RNA(short hairpinRNA, shRNA) 的DNA 模板设计3 条表达载体(shRNA255, shRNA554, shRNA593),并克隆到质粒pGPU6/GFP/Neo。经酶切和测序鉴定,筛选转染率最高基因沉默效果最好的shRNA 作RNA 干扰。DU145 细胞株分为空白质粒转染组和shRNA 转染组。转染前后按化学治疗药物分为氟尿嘧啶(fluorouracil,FU) 组及紫杉醇(paclitaxel,PA) 组,并按作用浓度(FU:30,60,120,240 μg/mL;PA:0.2,2,10,20 μg/mL) 分别分成4 个亚组,并分别设有一个空白对照组。四甲基偶氮唑盐[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide,MTT]比色法检测转染后细胞增殖活性的变化,MTT 及末端脱氧核苷酸转移酶介导脱氧尿苷三磷酸缺口末端标记法(terminal de-oxynucleotidyl transferasemediateddUTP nick end labeling,TUNEL) 检测转染前后不同浓度FU 和PA 对DU145 细胞抑制增殖及诱导凋亡的效果。结果:3 条表达载体(pGPU6/GFP/Neo-shRNA255, pGPU6/GFP/Neo-shRNA554, pGPU6/GFP/Neo-shRNA593) 的转染率分别为(63.3±1.04)%,(76.2±0.68)%,(72.7±0.33)%,shRNA554 转染率最高,三者之间比较差异有统计学意义(P<0.01)。转染后mRNA 含量分别为128.31±2.50,43.24±4.30 和85.62±6.30,GSTP1 蛋白含量分别为163.92±12.40,65.38±9.30和114.25±16.70,shRNA554 转染后GSTP1 基因mRNA 和蛋白含量均最低,三者比较差异有统计学意义(P<0.01)。MTT 检测转染前FU 不同浓度 (30, 60, 120, 240 μg/mL) 四个亚组作用后细胞存活率分别为(95.60±2.11)%,(90.20±0.86)%,(83.10±3.12)% 和(74.60±1.32)%;转染后存活率为(91.30±1.43)%,(84.6±2.13)%,(73.2±1.52)%和(65.5±0.94)%。TUNEL 检测转染前FU 不同浓度(30, 60, 120, 240 μg/mL) 四个亚组作用后细胞凋亡率分别为(5.50±0.88)%,(10.20±1.64)%,(15.20±2.39)% 和(25.10±2.59)%;转染后凋亡率(10.80±0.62)%,(15.70±1.32)%,(20.40±1.89)% 和(34.90±2.54)%。转染后相同FU 浓度下细胞存活率降低,凋亡率增加,差异均具有统计学意义(P<0.01)。MTT 检测转染前PA 不同浓度(0.2, 2, 10, 20 μg/mL) 四个亚组作用后细胞存活率为(98.50±2.34)%,(95.20±1.32)%,(89.40±0.68)%和(82.70±1.73)%;转染后存活率为(94.2±0.78)%,(86.5±2.13)%,(78.7±1.34)% 和(70.1±0.76)%。TUNEL 检测转染前PA 不同浓度 (0.2, 2, 10, 20 μg/mL) 四个亚组作用后细胞凋亡率分别为(2.4±1.07)%,(5.2±1.33)%,(10.5±2.41)%和(20.7±1.92)%;转染后凋亡率(5.46±2.13)%,(13.8±1.24)%,(21.2±2.39)% 和(29.2±2.21)%。转染后相同PA浓度下细胞存活率降低,凋亡率增加,差异均具有统计学意义(P<0.01)。结论:shRNA 介导的GSTP1 基因沉默可抑制雄激素非依赖型前列腺癌DUl45 细胞增殖活性,诱导凋亡,并且其作用呈现时间依赖性,可提高其对于化学治疗的药物敏感性。

关键词： 前列腺癌细胞株DU145 短发卡状RNA 谷胱甘肽S-转移酶P1 化学治疗敏感性

Effect of gene GSTP1 silencing via shRNA transfection on androgen independent prostate cancer cell line Du145

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Abstract: Objective: To design short hairpin RNA (shRNA) interference sequence to silence glutathione S-transferase P1 (GSTP1) gene of androgen independent prostate cancer cell line DU145, and to explore its effect on proliferation and sensitivity to chemotherapeutics. Methods: The target sequence was picked up to form the shRNA, and the 3 shRNA expression vectors were shRNA255, shRNA554 and shRNA593. The DNA template was cloned to plasmid pGPU6/GFP/Neo. The shRNA was identified by enzyme digesting and gene sequencing. The screening experiment was done to pick up the shRNA expression vector with the highest transfection ratio and best gene silencing results. DU145 cells were divided into a blank plasmid group and a shRNA transfected group. According to the chemotherapeutics the DU145 cells were divided into a fluorouracil (FU) group and a paclitaxel (PA) group, and the 2 groups were subdivided under different concentrations of PA (0.2, 2, 10, and 20 μg/mL) were (2.40±1.07)%, (5.20±1.33)%, (10.50±2.41)%, (20.70±1.92)%; after the transfection the apoptosis ratio of cells was (5.46±2.13)%, (13.80±1.24)%, (21.20±2.39)%, and (29.20±2.21)%. After the transfection, the cell survival ratio decreased under the same PA concentration, and the apoptosis ratio increased, with statistical significance (both P<0.01). Conclusion: gene GSTP1 silence via shRNA transfection to androgen independent prostate cancer cell line DU145 can inhibit its proliferation in time dependent manner, and induce apoptosis and raise its sensitivity to chemotherapeutics. into 4 subsets according to the chemotherapeutic concentrations (FU: 30, 60, 120, and 240 μg/mL; PA: 0.2, 2, 10, and 20 μg/mL),

meanwhile a blank control group was included respectively. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to evaluate the proliferation after the transfection. MTT and terminal de-oxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay were used to detect the inhibition effect of different concentrations of 5-FU or PA on the proliferation and induction of apoptosis of DU145. Results: The transfection ratio of the 3 shRNA expression vectors (shRNA255, shRNA554, and shRNA593) was (63.30±1.04)%, (76.20±0.68)%, and (72.70±0.33)%, and the transfection ratio of shRNA554 was the highest. there was significant difference among the above 3 shRNA expression vectors (P<0.01). After the transfection, the mRNA was 128.31±2.50, 43.24±4.30 and 85.62±6.30, the GSTP1 protein was 163.92±12.40, 65.38±9.30 and 114.25±16.70. After the transfection of shRNA554, the mRNA and protein of GSTP1 were the lowest level. there was significant difference among the above 3 shRNA expression vector (P<0.01). MTT analysis showed that before the transfection, the survival ratio of cells under different concentrations of FU (30, 60, 120, and 240 μg/mL) was (95.60±2.11)%, (90.20±0.86)%, (83.10±3.12)% and (74.60±1.32)%; however after the transfection, the survival ratio of cells was (91.30±1.43)%, (84.60±2.13)%, (73.20±1.52)%, and (65.5±0.942)%. TUNEL assay showed that before the transfection, the apoptosis ratio of cells under different concentrations of FU (30, 60, 120, and 240 μg/mL) was (5.50±0.88)%, (10.20±1.64)%, (15.20±2.39)%, and (25.10±2.59)%; however after the transfection, the apoptosis ratio of cells was (10.8±0.62)%, (15.7±1.32)%, (20.4±1.89)%, and (34.9±2.54)%. After the transfection, the cell survival ratio decreased under the same concentration of FU, and the apoptosis ratio increased, with statistical significance (both P<0.01). MTT analysis showed that before the transfection, the survival ratio of cells under different concentrations of PA (0.2, 2, 10, and 20 μg/mL) was (98.50±2.34)%, (95.20±1.32)%, (89.40±0.68)%, and (82.70±1.73)%; after the transfection the survival ratio of cells was (94.20±0.78)%, (86.50±2.13)%, (78.70±1.34)%, and (70.10±0.76)%. TUNEL assay showed that before the transfection, the apoptosis ratio of cells

Keywords: prostate cancer cell line DU145　short hairpin RNA　glutathione S-transferase P1　chemotherapeutic sensitivity

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